

# Positive Genetic Correlation between Forage Yield and Fiber of Smooth Bromegrass

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## ABSTRACT

Voluntary intake potential of a forage crop is generally considered to be the most important feed characteristic regulating animal performance. Efforts to develop forage crops with reduced bulk volume, measured by neutral detergent fiber (NDF) concentration, have been associated with reduced plant fitness. The objective of this study was to evaluate a second cycle of divergent selection for NDF in four smooth bromegrass (*Bromus inermis* Leyss.) pedigrees and to determine if concomitant selection for spaced-plant vigor could counteract the loss in forage yield associated with selection for low NDF. In both high and low directions of Cycle-2, populations were created with and without concomitant selection for plant vigor. Selection responses for NDF and forage yield were linear through both cycles of selection in both directions. Although responses were not uniform across the four pedigrees, changes in NDF were reflected in changes in forage yield on all levels evaluated: selection direction (high vs. low NDF), individual cycles, and vigor selections. Although combined selection for low NDF and high vigor improved forage yield in the low-NDF direction for one of four pedigrees, it did not alter the strong positive relationship between NDF and forage yield across all populations and cycles. The genetic correlation between NDF and forage yield appears to be physiological in origin, caused largely by overlapping genic specificities (i.e., pleiotropy).

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**Abbreviations:** C0, Cycle 0 (original populations); C-1, Cycle 1 low NDF; C-2, Cycle 2 low NDF; C-2V, Cycle 2 low NDF and high vigor; C+1, Cycle 1 high NDF; C+2, Cycle 2 high NDF; C+2V, Cycle 2 high NDF and high vigor; CL, Cycles linear; CQ, Cycles quadratic; CR, Cycles residual; NDF, neutral detergent fiber; P, pedigrees; V, vigor.

PLANT BREEDING EFFORTS to develop forage crops with improved intake potential are increasing (Casler and Vogel, 1999). While there has been considerable effort at making genetic improvements in digestibility (Casler and Vogel, 1999), most ruminant nutritionists consider voluntary intake to be more important than digestibility in limiting animal performance (Fahey and Hussein, 1999). Up to 70% of the variation in animal production can be attributed to variation in intake, while only 20% can be attributed to variation in digestibility (Crampton et al., 1960).

Neutral detergent fiber (NDF) is the most rapid and reliable laboratory predictor of voluntary intake potential of feeds (Mertens, 1994). Efforts to breed for increased intake potential of perennial grasses for ruminant livestock, by selection for reduced NDF concentration, have been plagued by persistent reductions in forage yield (Casler, 1999a; Han et al., 2001; Surprenant et al., 1988). Because NDF comprises the majority of plant cell walls (Van Soest, 1994; Casler and Hatfield, 2006), a positive genetic correlation between NDF and forage yield may be a biological necessity. One cycle of divergent selection for NDF in four diverse

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smooth brome grass (*Bromus inermis* Leyss.) pedigrees led to uniform positive correlated responses in forage yield (Casler, 2005). These results implied a significant proportion of the genetic control of forage yield derives from loci or linkage blocks with shared inheritance of forage yield and NDF. This could be due to either pleiotropy or very tight linkage between loci that independently control forage yield and NDF.

Combined selection for low NDF and high forage yield or spaced-plant vigor would provide a test of the hypothesis that the genetic correlation between forage yield and NDF is fixed and cannot be altered by short-term selection methods. Selection for increased forage yield on a spaced-plant basis has translated to increased sward-plot forage yields in several grasses, including switchgrass (*Panicum virgatum* L.) (Missaoui et al., 2005); Pensacola bahiagrass (*Paspalum notatum* Flugge var. *saure* Parodi) (Gates et al., 1999; Burton and Mullinix, 1998); Italian ryegrass (*Lolium multiflorum* Lam.) (Fujimoto and Suzuki, 1975); and cereal rye (*Secale cereale* L.) (Bruckner et al., 1991). Vogel and Pedersen (1993) described routine selection methodology in which spaced-plant nurseries are prescreened for forage yield and other agronomic traits and only those plants with superior forage yield are sampled and evaluated for forage quality traits. The objective of this study was to conduct and evaluate a second cycle of divergent selection for NDF in four smooth brome grass pedigrees, with and without concomitant selection for spaced-plant vigor as a means of ameliorating the loss in forage yield associated with selection for low NDF.

## MATERIALS AND METHODS

Phenotypic selection was applied to four smooth brome grass populations (pedigrees): the cultivars Alpha and Lincoln, and the synthetic populations WB19e and WB88S-Alt (Falkner and Casler, 1998). Cycle-1 high-NDF and low-NDF progeny populations were created in each base population. The selection protocol was described in detail by Diaby and Casler (2005). Briefly, two cycles of selection were completed in each of the high and low directions for NDF, using phenotypic selection on a grid system. Each nursery consisted of 350 plants in 10 grids of 35 (five rows by seven columns). Ten plants were selected from each nursery for highest or lowest NDF and adjusted for block-to-block variation. Cycle-1 was conducted in 1992 to 1995 and Cycle-2 was conducted in 1996 to 1999. Selected plants were cloned and transplanted to isolated polycross blocks (Diaby and Casler, 2005).

Plants were selected in Cycle-2 based on two selection criteria: (i) vigor and NDF using independent culling levels and (ii) NDF alone. First, each plant was visually scored for vigor, using four independent raters and a rating scale of 1 = unacceptable, 2 = marginally acceptable, or 3 = acceptable. Vigor ratings were made on vegetative growth in mid-May (immediately before sampling plants for NDF determination), late July, and early October 1997. Visual vigor ratings have

been used as a successful proxy for spaced-plant forage yield in several species (Casler et al., 1998; Casler and van Santen, 2000; Casler, 2001). The 12 vigor scores were averaged and used to rank the 350 plants in each nursery for mean vigor, using the top 100 plants as the source material for selection of the 10 plants with highest or lowest NDF. Second, the 10 highest and lowest in NDF were selected from each population were selected, exactly as described by Diaby and Casler (2005), from among all 350 plants. Cycle-2 selection generated a total of 16 groups of 10 plants: eight nurseries (Cycle-1 high or low of Alpha, WB19e, Lincoln, and WB88S)  $\times$  two selection criteria (NDF or NDF + vigor).

All 28 populations were planted in 0.9 by 3.0 m plots at three locations in April 2003. Locations and soil types were: Arlington, WI (43°20' N, 89°23' W; Plano silt loam [fine-silty, mixed, mesic Typic Argiudoll]), Marshfield, WI (44°40' N, 90°10' W; Withee silt loam [fine-loamy, mixed, superactive, frigid Aquic Glossudalf]), and Lancaster, WI (42°50' N, 90°47' W; Fayette silt loam [fine-silty, mixed, mesic Typic Hapludalf]). The experimental design was a split-plot in randomized complete blocks with four replicates, in which the four original germplasm pedigrees were whole plots, and the seven cycles (C-2V, C-2, C-1, C0, C+1, C+2, and C+2V, where V = vigor) were subplots. The seeding rate was 21 kg ha<sup>-1</sup> on a pure-live-seed basis. Germination of each population and cycle was determined according to standardized procedures (Association of Official Seed Analysts, 1998). Plots were clipped twice during the establishment year and fertilized once with 56 kg N ha<sup>-1</sup>.

Plots were harvested with a flail harvester three times per year in 2004 through 2006, generally in early June, early August, and October. Each location was fertilized with 90 kg N ha<sup>-1</sup> in early spring and following each of the first two harvests of each year. A random 500-g sample was collected from the harvested forage of each plot and dried at 60°C for dry matter determination. Forage yield was summed over harvests within each year before any statistical analysis.

Dry samples were ground through a 1-mm screen of a Wiley-type mill and scanned by near-infrared reflectance spectroscopy. Cluster analysis of reflectance spectra was used to develop a subset of 96 samples for calibration development (Shenk and Westerhaus, 1991). The concentration of NDF for the 96 calibration samples was determined using the procedure of Van Soest et al. (1991), omitting sodium sulfite and  $\alpha$ -amylase. Calibration statistics for NDF were:  $R^2 = 0.96$ , standard error of calibration = 6.2 g kg<sup>-1</sup>, and standard error of validation = 7.9 g kg<sup>-1</sup>.

Forage yield was analyzed by nearest neighbor analysis for each location-year combination using the pre-adjustment of total forage yield method of Smith and Casler (2004). Spatially adjusted values of forage yield were analyzed by analysis of variance using the split-plot-in-time model (Steel et al., 1996). Degrees of freedom in these models were subtracted from pooled experimental errors, according to the number of parameters fit in the spatial models for each location-year combination (Casler, 1999b). Experimental error mean squares and all *F*-tests were recomputed in a spreadsheet after adjustment of error degrees of freedom. All effects were fixed, except replicates, which were assumed to be random. Data for NDF were used to compute

weighted means across harvests within a season, using forage yields of each harvest as the weighting factors (Casler, 1999a, 2005). Weighted seasonal NDF was analyzed using the same multilocation and multiyear model as applied to forage yield data.

Selection responses were computed for both NDF and forage yield, using contrasts to estimate linear, quadratic, and residual selection responses across cycles. The full partition of sum of squares for the 28 populations was as follows: pedigrees (P, 3 df), Cycles linear (CL, 1 df), Cycles quadratic (CQ, 1 df), Cycles residual (CR, 2 df),  $P \times CL$  (3 df),  $P \times CQ$  (3 df),  $P \times CR$  (6 df), vigor (V, 1 df),  $V \times P$  (3 df),  $V \times CL$  (1 df), and  $V \times CL \times P$  (3 df). In addition, paired Cycle-2 populations derived by selection for NDF alone or NDF + V were compared using contrasts.

## RESULTS AND DISCUSSION

Population  $\times$  location, population  $\times$  year, and population  $\times$  location  $\times$  year interactions were not significant for NDF (Table 1). All three interactions were significant for forage yield (Table 1;  $P < 0.05$ ), but combined together, accounted for only 13.3% of the variance of a population mean. Therefore, for both NDF and forage yield, results and conclusions did not vary significantly across locations or years and means over three locations and three years were used for all data presentations and discussions.

For NDF, most of the variation among the 28 populations could be attributed to linear selection responses (Table 1). As reported previously, NDF has a moderate heritability in smooth bromegrass, results of selection in spaced-plant nurseries are highly predictable for sward plots, and selection for divergent NDF of leaf blades is robust with respect to harvest dates, locations, and years (Casler, 1999a, 2005). Unlike observations made from one cycle of divergent selection for NDF (Casler, 2005),

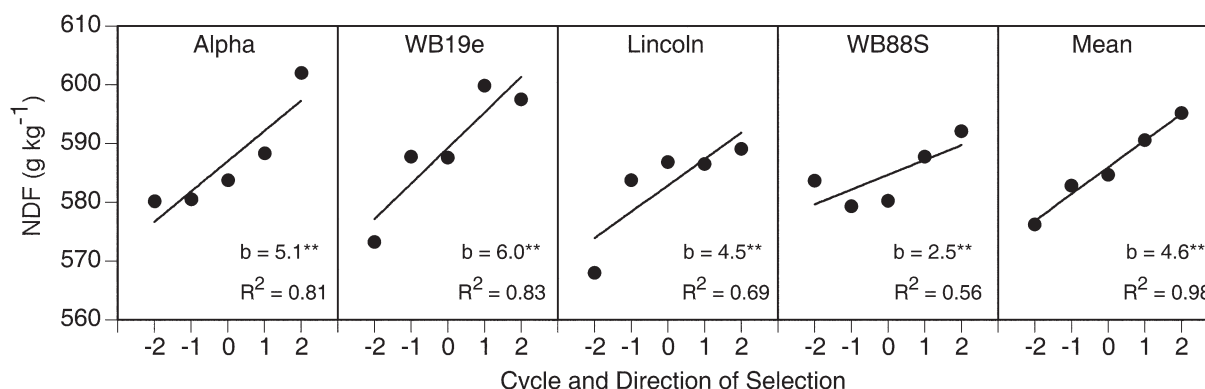
**Table 1. Partial analysis of variance partition for neutral detergent fiber (NDF) and forage yield measured on 28 smooth bromegrass populations representing seven populations derived from divergent selection for NDF or NDF plus vigor within each of four original source populations (pedigrees).**

Source of variation	df	NDF			Forage yield		
		MS	SS <sup>†</sup>	P value	MS	SS <sup>†</sup>	P value
			%			%	
Pedigrees (P)	3	2145	10.4	<0.0001	89.88	53.9	<0.0001
Cycles linear (CL)	1	41284	66.6	<0.0001	118.05	23.6	<0.0001
Cycles quadratic (CQ)	1	17	0.0	0.6922	0.23	0.0	0.6422
Cycles residual (CR)	2	211	0.7	0.1465	3.36	1.3	0.0427
$P \times CL$	3	985	4.8	<0.0001	5.63	3.4	0.0014
$P \times CQ$	3	2061	10.0	<0.0001	7.03	4.2	0.0002
$P \times CR$	6	313	3.0	0.0101	8.72	10.5	<0.0001
Vigor (V)	1	190	0.3	0.1874	0.48	0.1	0.5002
$V \times P$	3	361	1.7	0.0205	2.74	1.6	0.0525
$V \times CL$	1	936	1.5	0.0037	0.56	0.1	0.4659
$V \times P \times CL$	3	191	0.9	0.1566	1.92	1.2	0.1426
Population $\times$ location	54	132		0.1642	1.67		0.0106
Population $\times$ blocks/location	234	109		0.1816	1.05		0.0099
Population $\times$ year	54	178		0.1092	2.44		0.0000
Population $\times$ location $\times$ year	108	116		0.0638	0.93		0.0278
Pooled error	495	93			0.71		

<sup>†</sup>Sum of squares percentages included only the 27 df for the main effect of populations (sum = 100%).

results were not consistent across the four pedigrees. Differences in selection responses accounted for 17.8% of variation among the 28 populations in the experiment (Table 1). Some of this variation could be attributed to differences in linear response among the four pedigrees (Fig. 1), but a large proportion was due to changes in quadratic responses or to apparently random variation. The quadratic and residual components of the pedigree  $\times$  cycle interaction were most likely due to genetic drift (Falconer, 1953; Casler, 2005).

For forage yield, pedigree accounted for over half of the variation among the 28 populations, while the average linear selection response accounted for about one quarter of the variation (Table 1). On average, each cycle of selection for reduced NDF decreased forage yield by 0.21 Mg ha<sup>-1</sup>.



**Figure 1.** Graphical representation of direct selection responses for neutral detergent fiber (NDF) following two cycles of divergent selection for NDF within four different smooth bromegrass pedigrees (b, linear selection response; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ).

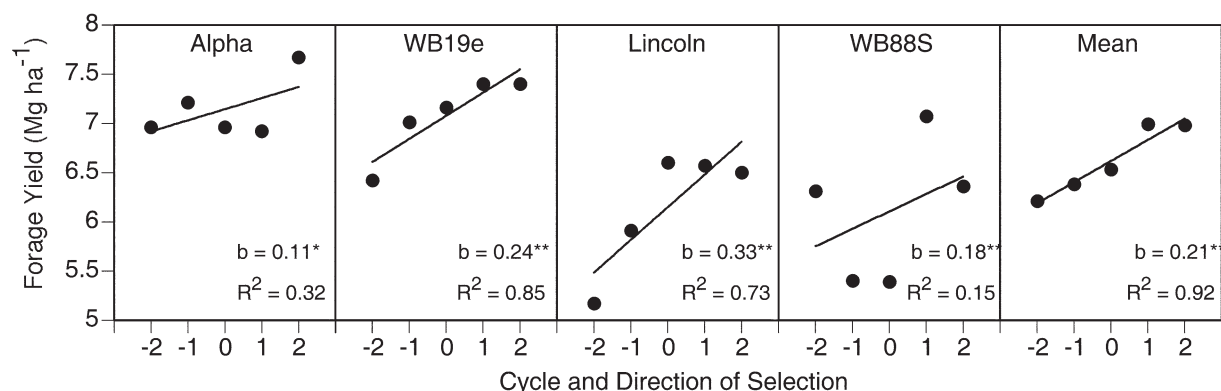


Figure 2. Graphical representation of correlated selection responses for forage yield following two cycles of divergent selection for neutral detergent fiber (NDF) within four different smooth brome grass pedigrees (b, linear selection response; \* $P < 0.05$ ; \*\* $P < 0.01$ ).

However, this effect was quite variable across the four pedigrees, ranging from 0.11 to 0.33  $\text{Mg ha}^{-1}$  (Fig. 2). Variation among the four selection responses accounted for 18.1% of the overall population variation (Table 1) and clearly indicated that forage yield selection responses could not be predicted directly from NDF selection responses. The phenotypic correlation between NDF and forage yield across the 28 populations was  $r = 0.73$  (Fig. 3;  $P < 0.01$ ), but the phenotypic correlation between linear selection responses for NDF and forage yield (Fig. 1 and 2) was only  $r = 0.10$ .

The random effects of drift could explain some of this lack of relationship in selection responses, but a large amount of symmetry in selection responses for NDF and forage yield (Fig. 1 and 2) belies such a simple explanation. For example, WB88S had the least predictable selection response for both NDF and forage yield, with the lowest realized gains for NDF and the lowest  $R^2$  value for both variables. Despite this, the phenotypic correlation coefficient between forage yield and NDF of the seven

WB88S populations was  $r = 0.79$  ( $P < 0.01$ ). Lincoln had the largest quadratic effect, which equates to asymmetry of selection response between the high and low directions, for both NDF and forage yield. For both variables, selection was considerably more effective in the low-NDF direction. For WB19e, selection for high NDF in Cycle-2 was ineffective and the lack of change in forage yield for C+2 reflected this. For Alpha, the largest individual NDF selection response was for high NDF in Cycle-2, which was also reflected in the largest individual forage-yield selection response.

Selection for vigor had significant ( $P < 0.05$ ) effects in four of eight cases for NDF and two of eight cases for forage yield (Table 2). In the low-NDF direction (C-2), selection for vigor resulted in reduced selection response in NDF in WB19e and Lincoln, but greater selection response in WB88S. Two of these responses, WB19e and WB88S, were positively associated with changes in forage yield. In the high-NDF direction (C+2), selection for vigor had only one effect, to reduce the selection response for NDF in WB88S.

Selection for vigor was successful in reducing the loss in forage yield associated with low NDF in only one of four pedigrees. This could be due to relatively low heritability of vigor ratings in smooth brome grass and/or a low genetic correlation between spaced-plant vigor rating and sward-plot forage yield. Taken together, the positive forage-yield response in WB19e and the negative forage-yield response in WB88S suggest that responses of forage yield to selection for vigor were regulated more by random processes, such as drift, than by systematic processes, such as selection on genes for forage yield.

The positive correlations of NDF with forage yield in these two responses to selection for vigor reinforced the overall positive genetic correlation between these two traits (Fig. 3; Table 2). The remaining two responses of NDF to selection for vigor both worked against the selection goal of low NDF (C-2V Lincoln) or high NDF (C+2V WB88S), suggesting that the reduced population size (100 vs. 350), selection intensity, and selection differential, and perhaps

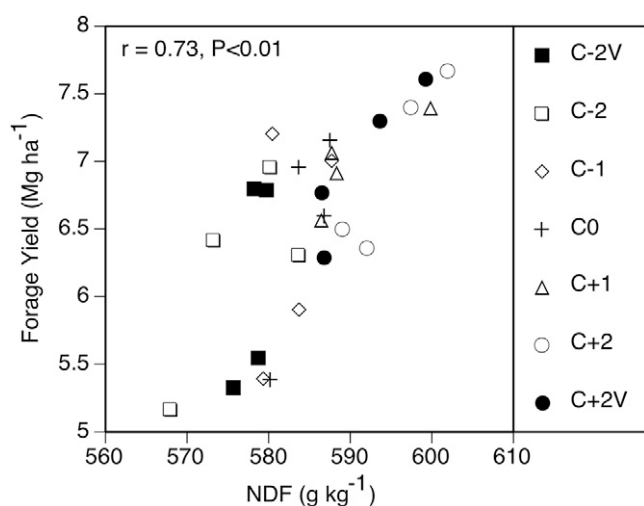


Figure 3. Scatterplot of mean forage yield vs. neutral detergent fiber (NDF) concentration measured on 28 populations created by divergent selection for NDF combined with selection for high vigor in a subset of populations (C0, Cycle 0, original populations; C-1, Cycle 1 low NDF; C+1, Cycle 1 high NDF; C-2, Cycle 2 low NDF; C+2, Cycle 2 high NDF; V, selection for high vigor).



random effects of drift, were also responsible for some of the NDF responses to selection for vigor. These results were very much like those of Surprenant et al. (1988) for reed canarygrass (*Phalaris arundinacea* L.) in which combined selection for NDF and forage yield served largely to reduce the gains to be made for NDF, partly by the positive genetic correlation between the two traits and partly by the restricted selection differential for NDF resulting from a smaller population size. Selection for low NDF led to a wide range of correlated responses in forage yield, but none of these derived populations had forage yield equal to the best populations selected for high NDF (Fig. 3).

## CONCLUSIONS

Partitioned selection responses suggested that most of the variation in forage-yield selection responses was likely due to a common set of loci or tight linkage groups that contain loci regulating NDF (Casler, 2005). Although NDF is not a specific biological entity, but a residue following solubilization in neutral detergent, it is likely controlled by a large number of loci that are involved in lignin, cellulose, and arabinoxylan synthesis and polymerization. The coincidence of forage yield and NDF selection responses is difficult to ignore, strongly implying a physiological relationship between NDF and forage yield that is, in large part, unalterable. Neutral detergent fiber comprises the majority of the cell wall in smooth brome grass, with the exception of a few minor components that are soluble at neutral pH (Casler and Hatfield, 2006). The plant cell wall represents a physical frame on which numerous plant functions and processes are built. Cell walls are responsible for the retention of upright growth as tillers grow taller, larger, and heavier. Cell walls also function in the transport of nutrients, photosynthate, and water through the vascular system of a tiller. Older phytomers have higher NDF concentrations (Kephart et al., 1990), suggesting an evolutionary adaptation to maintain upright tillers in the grass canopy. These functions of cell walls all allow the plant to continue accumulating dry matter, assuming that no other physiological functions become limiting.

The genetic correlation between NDF and forage yield appears to be physiological in origin, caused largely by overlapping genic specificities (i.e., pleiotropy) (Casler, 2005). There is a strong positive genetic correlation between NDF measured on both spaced plants and sward plots. Although the genetic correlation between spaced-plant vigor and sward-plot forage yield was not strong, both traits were positively correlated with NDF. Alternative breeding schemes, based on measurement of forage yield on sward plots and NDF based on spaced plants, will not alter the results of this study. Forage yield and NDF are not exactly proxies for each other, but their genic specificities overlap to such a strong degree that alterations in one trait will inevitably bring some level of positive

**Table 2. Mean neutral detergent fiber (NDF) and forage yield of Cycle-2 populations selected for low NDF (C-2), high NDF (C+2), low NDF plus high vigor (C-2V), or high NDF plus high vigor (C+2V) within four source populations of smooth brome grass.**

Variable/ source population	Cycle-2 low-NDF		Cycle-2 high-NDF	
	NDF (C-2)	NDF + vigor (C-2V)	NDF (C+2)	NDF + vigor (C+2V)
NDF	g kg <sup>-1</sup>			
Alpha	580.2	579.8	602.0	599.2
WB19e	573.3	578.3*	597.5	593.6
Lincoln	568.0	575.7**	589.1	586.5
WB88S	583.7	578.8*	592.1	586.8**
Forage yield	Mg ha <sup>-1</sup>			
Alpha	6.96	6.79	7.67	7.61
WB19e	6.42	6.90*	7.40	7.30
Lincoln	5.17	5.33	6.50	6.77
WB88S	6.31	5.55**	6.36	6.29

\*Means of the two selection criteria (NDF alone vs. NDF + vigor) are different at  $P < 0.05$ .

\*\*Means of the two selection criteria (NDF alone vs. NDF + vigor) are different at  $P < 0.01$ .

correlated response for the other trait. Low NDF does not appear to be a desirable mechanism to facilitate genetic improvements in intake potential of forage grasses.

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